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Staining of wool using the reaction products of ABTS oxidation by Laccase: Synergetic effects of ultrasound and cyclic voltammetry

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Abstract

The effects of ultrasound on 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonate) enzymatic oxidation by laccase (*Trametes villosa*) has been studied by means of cyclic voltammetry. The reaction was allowed to proceed in the presence of a piece of wool and the coloration depth of the wool fabric was measured by means of *K*/*S*. It was observed that cyclic voltammetry is influenced the dyeing process and higher *K*/*S* values were obtained when the cyclic voltammetry was combined with the ultrasonic irradiation. Moreover, the *K*/*S* value is the sum of the values obtained when the wool staining is done in just the presence of cyclic voltammetry or in just the presence of ultrasound.

The results obtained on the indigo carmine decolourization gives information on the importance of controlling the amount of $ABTS^{\cdot+}$ formed during the ultrasonication process.

Keywords: ABTS; Laccase; Cyclic voltammetry; Ultrasound; Wool; Indigo carmine decolourization

1. Introduction

Laccase is the generic name given to a family of multi-copper oxidases that are able to catalyze the oxidation of phenolic compounds and of other aromatic compounds with concomitant reduction of oxygen to water [1,2]. As demonstrated by Palmore and Kim [3], as well as being a good substrate, ABTS, can be an excellent electrochemical mediator for laccases.

ABTS, undergoes reversible oxidation to form a stable and intensively colored cation radical $ABTS^{\cdot+}$ and the dication $ABTS^{2+}$ (Fig. 1). In the literature are reported different opinions regarding the formed reaction products of the oxidation of ABTS by laccase. Some authors sustain the idea that it can be generated by just the cation radical [4,5], while others that report the formation of both the cations are produced by the laccase action on ABTS [6,7]. To the best of our knowledge, the color of the $ABTS^{2+}$ species is ambiguously described elsewhere in the literature. For example, Bourbonnais et al. [6], suggested that $ABTS^{2+}$ is purple while Barreca et al. [8], suggested that $ABTS^{2+}$ is red.

Ultrasound as a means of intensification of wet textile processes has been attempted by several researchers in the past few years [9–13]. Meanwhile, power ultrasound can enhance a wide variety of chemical and physical processes mainly due to the phenomenon of cavitation in a liquid medium that is the growth and explosive collapse of microscopic bubbles. Sudden and explosive collapse of these bubbles can generate "hot spots" [14,15], i.e., localized high temperature, high pressure, shock waves and severe shear force capable of breaking chemical bonds.

In this paper the results of the investigation of the effect of 20 kHz ultrasound on ABTS oxidation by laccase are

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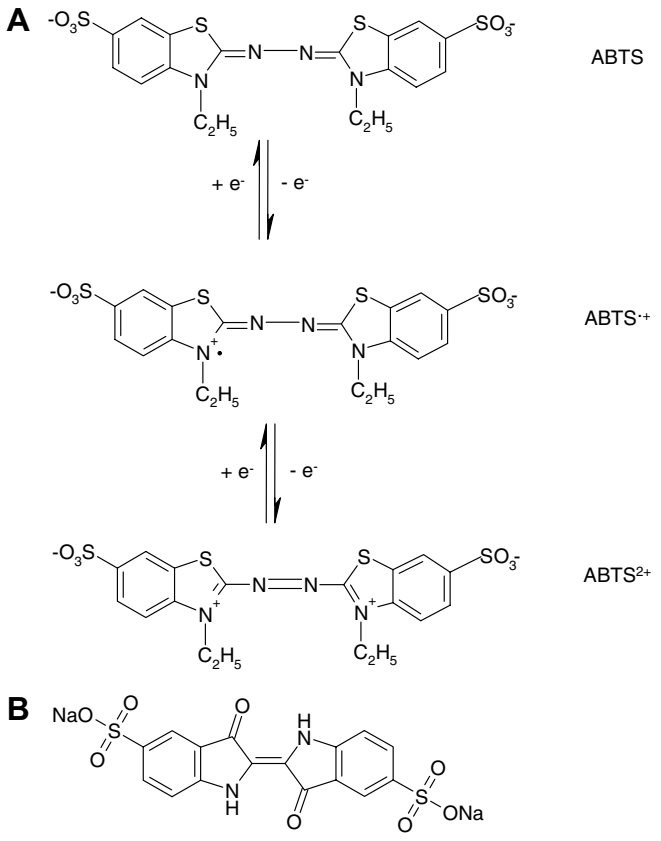

Fig. 1. (A) Formation of the cation radical and the dication by removal of one and two electrons from ABTS and (B) structure of indigo carmine.

discussed, as well as the results obtained when wool fabric was present in the bulk solution. It was observed that the cyclic voltammetry has a synergetic effect on wool staining when coupled with the ultrasound. In continuation of our interest is the understanding of the effects of ultrasound and/or enzymes on the textile fabric treatment.

2. Material and methods

2.1. Chemicals

Fungal laccase (benzenediol/oxygen oxidoreductase: EC 1.10.3.2) from *Trametes villosa* was kindly supplied by Novo Nordisk (Denmark) and stored at 4 °C. This was used as received without doing any previous dilution. 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and indigo carmine (Fig. 1B) were purchased from Sigma (Spain) and used as received. All the other chemicals were of reagent grade. Solutions of the different mediators were prepared in 0.1 M acetate buffer pH 5 before use.

The wool (Albano Antunes Morgado Lda, Castanheira de Pera, Portugal) used in the experiments was previously washed with Lutensol AT-25 (BASF, Ludwigshafen, Germany) (non-ionic surfactant) 1 g/l, in a bath ratio 1:20 at pH 9.0 ($NaHCO_3$ 0.1 M buffer), for 30 min, at 40 °C, on a Rota-wash (MKII Series 7227, from the Shirley Developments Limited, Stockport, England). After the washing procedure, the surfactant was removed from fabric first with tap water, followed by washing with distilled water.

2.2. Electrochemical experiments

The cyclic voltammetry experiments were performed using a Voltalab 30 Potentiostat (Radiometer Analytical, France), controlled by the Voltamaster 4 (version 5.6) electrochemical software. The working, counter and reference electrodes were respectively: glassy carbon (0.07 cm^2), coiled platinum wire (23 cm) and an Ag|AgCl electrode filled with saturated KCl (BAS, Bioanalytical Systems, West Lafayette, IN, USA). The supporting electrolyte used in the electrochemical cell was a solution of 0.1 M acetate buffer pH 5.

The ultrasonic irradiation was carried out with the equipment Sonics & Materials (USA) operating at 20 kHz (6 W/cm^2). The diameter of the glass cell was 60 mm and the height was 200 mm. The glass cell was not sealed, and cold water was circulated around the cell in order to maintain a constant temperature. The cylindrical sonochemical reactor (volume 150 ml) was thermostated by a water jacket.

The ultrasound horn (probe diameter 13 mm) was parallel to the working electrode and situated at a distance of approximately 3 cm away from this.

The stirred experiments were conducted in the same reactor but the ultrasound horn was replaced by a Teflon coated magnetic bar rotating at 500 rpm.

2.3. K/S measurements

Color depth was evaluated in terms of K/S values and these were calculated using Kubelka–Munk's equation ($K/S = (1 - R)^2/2R$, where R is the reflectance). For each fabric sample, five reflectance measurements were made and the arithmetic mean of these determinations was used for the K/S. The reflectance values were measured with a Datacolor apparatus at standard illuminant D65 (LAV/Spec. Incl., d/8, D65/10°).

Before the measurement of the K/S values the samples were washed with tap water. The removal of the no covalent bound product from the fibres was done in a Rota-Wash machine using the washing agents Lutensol AT-25 (1:100) at a temperature of 50 °C for 45 min. The samples were left to dry at room temperature.

3. Results and discussion

3.1. Cyclic voltammetry of ABTS in the absence and in the presence of ultrasonic irradiation

The electrochemistry of ABTS was tested at a glassy carbon working electrode by means of cyclic voltammetry at a scan rate of 20 mV/s as the obtained cyclic voltammograms are shown in Fig. 2. The bulk solution consisted of 1 mM ABTS solution prepared in 0.1 M acetate buffer pH 5.0 and the final volume was 150 ml. In this case when the

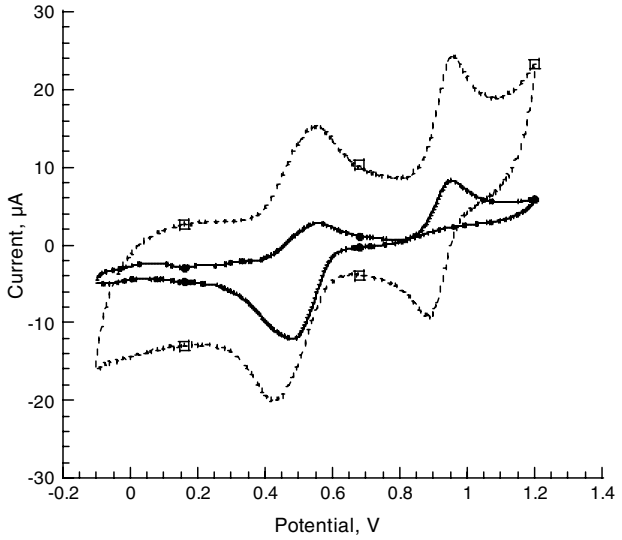

Fig. 2. Cyclic voltammograms of ABTS in the presence of laccase at scan rate of 20 mV/s, dashed line in absence of ultrasound, continuous line in the presence of ultrasound.

experiments were performed in the presence of laccase, to the bulk solution was added 100 μl laccase.

At the working pH (pH 5.0) the ABTS is completely deprotonated. As can be seen from Fig. 2, in quiet solution two oxidation peaks were observed corresponding to the oxidation of ABTS to its cation radical $ABTS^{\cdot+}$ and subsequently to its dication $ABTS^{2+}$, and the reverse scan shows two cathodic peaks corresponding to the reduction of the dication and of the cation radical, respectively.

In the same figure can be observed the cyclic voltammograms obtained in the presence of ultrasound irradiation. It can be observed that in this case the "limiting current" is diminished, due to the fact that in this case the mass transport of the active species is no more entirely controlled by diffusion. However in the presence of ultrasonic irradiation the cyclic voltammograms present well shaped oxidation peaks as obtained in the silent solution, but only one cathodic process was observed. This behavior can be possibly related to the comproportionation reaction between ABTS and $ABTS^{2+}$ with the formation of the cation radical, $ABTS^{\cdot+}$, which has as effected the disappearance of a cathodic peak due to the reduction of $ABTS^{2+}$. This comproportionation reaction depends on the concentration of the active species close to the surface of the electrode, so that in the case when the concentration of ABTS is high, the comproportionation reaction of the dication will take place before its reduction at the electrode surface. When the system is exposed to ultrasonic irradiation the comproportionation reaction will be favored as it is showed in the cyclic voltammograms obtained in the presence of ultrasound.

3.2. Coloration of different textile fabrics

Experiments were performed in order to check the affinity of the reaction products (obtained from the ABTS enzymatic oxidation) for different textile fibers (Fig. 3). As can be seen from this figure, the presence of wool effects a decrease in the reduction peak intensity, a fact that is not observed in the case when the other textile fabrics are used.

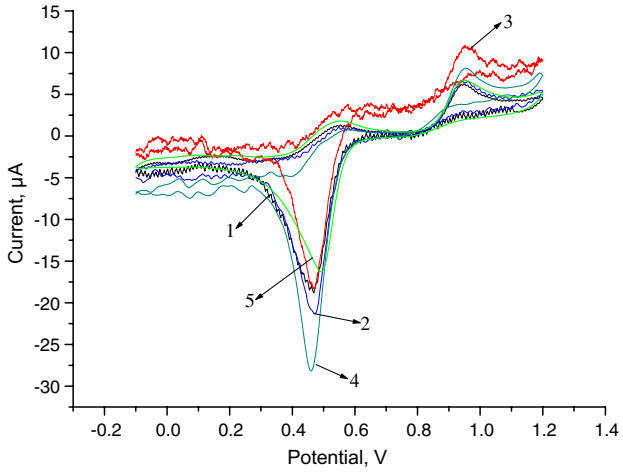

Fig. 3. Cyclic voltammograms of the ABTS enzymatic oxidation in presence of different textile fabrics (1 – cotton; 2 – polyacrilic; 3 – polyamide; 4 – polyester and 5 – wool).

After the removal of the textiles from the solution no colorations of the fabrics were observed in the case of polyacrilic, polyesteric and polyamidic fibres, while in the case of the wool and of the cotton a purple coloration of the fabric was detected. It has to be mentioned that in the case of the cotton fabric the purple coloration immediately turned to green after exposure to air, while the wool kept its purple coloration even after a long time exposure to air and/or washing in an aqueous solution of Lutensol AT-25.

3.3. Wool dyeing

In Fig. 4 are presented the results obtained using different approaches for enzymatic ABTS oxidation, and as can be seen from this figure different depths of the wool coloration can be achieved. The *K*/*S* value was employed to evaluate the color depth for the wool fabrics and it can

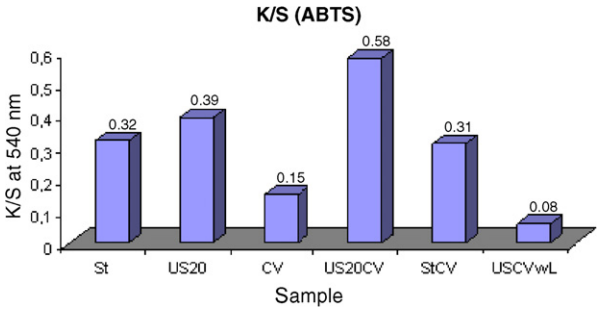

Fig. 4. *K*/*S* values of the wool dyed with $ABTS^{2+}$ under stirring (St), ultrasound (US20), cyclic voltammetry in quiet solution (CV), cyclic voltammetry and ultrasound (US20CV), cyclic voltammetry and stirring (StCV), and cyclic voltammetry and ultrasound but in absence of laccase (USCVwL).

be concluded that the ABTS enzymatic oxidation in a system where cyclic voltammetry is performed together with the ultrasonic irradiation gives a K/S value that is the sum of the K/S values obtained with the cyclic voltammetry and ultrasonic irradiation alone. Surprisingly not the same cumulative value is obtained in the case where the dyeing of the wool is obtained under continuous stirring and cyclic voltammetry. This result can be explained by taking into account the fact that the presence of the ultrasound given an advantage of improving the mass transport of the active species to/from the working electrode and a higher amount of $ABTS^{2+}$ is formed. At the same time this can be easily transported to the wool surface having as result the wool coloration. In order to confirm that the wool coloration is due to the reaction products of the enzymatic ABTS oxidation, blank experiments were performed having just the ABTS and wool in the system and exposing the system to ultrasound irradiation and running the cyclic voltammetry experiments at the same scan rate as in the other experiments. As it was expected the wool coloration was very low with a K/S value of 0.06.

3.4. Decolourization of Indigo Carmine

The laccases as well as laccase mediator systems may be used in biopulping, biobleaching, removal of toxic phenolic compounds from aquatic and terrestrial systems, synthesis of useful polymers by reticulation of phenolic compounds, degradation of lignite and hard coal structures, as analytical tools and as biosensors to estimate the quantity of phenols in natural juices or the presence of other enzymes [16,17].

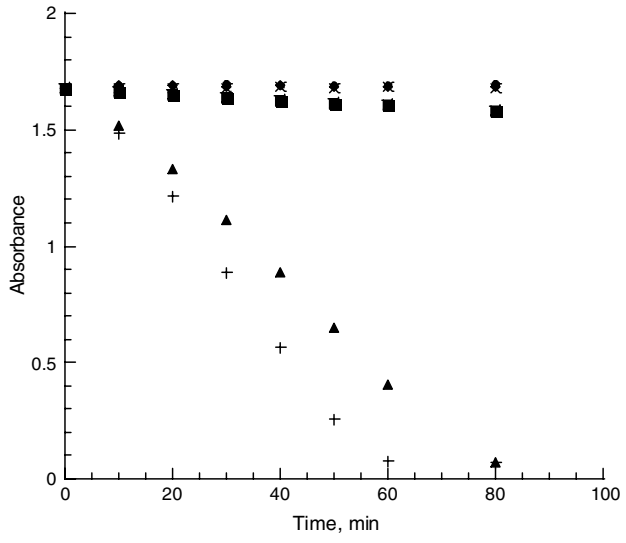

Fig. 5. Indigo carmine decolourization using the mediated or non-mediated system (● – control; ■ – decolourization in presence of laccase; ◆ – decolourization in the presence of ABTS; × – decolourization in the presence of ultrasonicated ABTS; + – decolourization in the presence of ABTS and laccase and ▲ – decolourization in the presence of ultrasonicated ABTS and laccase).

In the present study, the ability of the *T. villosa* laccase to decolorize Indigo Carmine (a commercial dye which is extensively used) was assessed. The decolourization of Indigo Carmine by laccase was performed in a mediated (ABTS) and unmediated system. Moreover, the experiment was also performed in order to check the influence of the formation of higher amounts of $ABTS^{2+}$ and $ABTS^{\cdot+}$ on the indigo carmine degradation by laccase.

150 ml of 1 mM solution of ABTS were exposed to ultrasonic irradiation of 20 kHz for 30 min (20 W/cm^2) thereafter 100 μl of the solution was removed and added to the 50 ml of indigo carmine 0.01 g/l solution. In the case when the degradation was done in the presence of laccase to the solutions were added 10 μl of laccase.

The amount of radicals formed during the ultrasonication process will affect the rate of decolourization of indigo carmine in the case of mediated process. As can be seen from Fig. 5 the decolourization rate of indigo carmine is much faster in the presence of ABTS and laccase, and the fact that in the case that the reaction of decolourization in the presence of a solution of ultrasonicated ABTS tends to be slower, it might be due to the formation of a higher amount of the ABTS dication in the disfavor of the formation of the ABTS cation radical.

4. Conclusions

The results obtained in the present work show the potential of ultrasound for the intensification of chemical/biochemical processes. The physical mass transport is improved and this is reflected also by the color depth obtained in the experiments where wool fabrics were dyed. Coupling the ultrasound with cyclic voltammetry will lead to a better dyeing of the wool due to the fact that in the presence of an ultrasound the formed $ABTS^{2+}$ species will be transported much faster from the electrode surface to the wool. In this way a higher color depth is obtained.

The experiments on decolourization of Indigo Carmine gives informations on the reaction rate of the ABTS enzymatic oxidation and it can be concluded that the ultrasonic irradiation of the laccase assisted oxidation of ABTS leads to the formation of higher amount of $ABTS^{2+}$ which will disfavor the decolourization of dye effluents if this is not done in the same reactor with the ultrasound.

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